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(57) Abstract

A bread improver comprises a latent enzyme preparation which is active during and after proving but relatively inactive during mixing.

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ENZYME-BASED BREAD IMPROVERS

The present invention relates to bread improvers, to enzyme preparations for use in bread improvers and to processes for preparing and using them. In particular, the invention relates to latent enzyme systems or preparations and their application in bread improver compositions and in the baking industry.

Bread is made from four principal ingredients: flour, yeast, salt and water. It is usually prepared in three basic steps, and the end result is a baked loaf. The steps are: (1) the principal ingredients are mixed to form a dough and worked to develop a continuous visco-elastic gluten matrix; (2) the developed dough is then proved by incubation in warm, humid conditions to promote fermentation by the yeast causing the dough to rise; (3) the risen dough is then baked to gelatinize starch, denature protein and fix the dough structure.

Various additives are known to improve dough development and the quality of the baked loaf. These are known as bread (or flour or dough) improvers/conditioners, referred to herein as "bread improvers". Bread improvers may include oxidants (such as ascorbic acid), reducing agents (such as cysteine), fats, emulsifiers, anti-moulding agents, yeast foods and enzymes. A typical bread improver consists of enzymes (0.1-2.0%), ascorbic acid (0.3-2.0%), fat/emulsifier (10-30%), soya flour (0-99%), anti-moulding agents (0-40%) and gypsum (0-40%), and is added to between 0.25 and 5% by weight of flour in the dough.

The enzymes which are used in improvers (referred to herein as "improver enzymes") include degradative enzymes (especially carbohydrases such as amylases, hemicellulases, pentosanases, pullulanases, xylanases and pectinases, but also lipases), redox enzymes (such as ascorbic acid oxidase, glucose oxidase) and oxygenases (such as lipoxygenase). Improver enzymes play a crucial rôle in determining the rheological, structural and compositional properties of the dough during mixing and proving, and have a dramatic impact on the quality of the bread produced.

For example, carbohydrases hydrolyse carbohydrates (such as polysaccharides) into smaller subunits (such as glucose and dextrins) which are used by the yeast as substrates for fermentation. They promote a soft, white crumb.

The starch-degrading enzyme α-amylase (and in particular fungal α-amylase, referred to herein as "FAA") is used in large quantities in the baking industry, and breaks down starch into glucose and dextrins. It is extremely effective in improving loaf volume, crumb whiteness,

softness of crumb, surface colour and keeping qualities. Various pentosanases have similar effects, particularly in improving loaf volume and shape.

However, certain problems are associated with the use of improver enzymes. These problems stem from the fact that hydrolytic enzymes reduce the bulk molecular weight of the carbohydrate fraction of the dough while increasing the concentration of relatively small molecular weight carbohydrates (such as sugars, glucose, oligosaccharides and dextrins). This results in a decrease in water holding capacity of the dough and the production of stickiness (resulting from solutions of the relatively small molecular weight hydrolytic products). α-amylases in particular tend to soften dough quite significantly and produce stickiness (especially at higher water levels). The consequences are lower yield (since less water can be added to the dough) and mixing difficulties.

There is therefore a need to provide improver enzymes (and bread improvers based thereon) which do not reduce the water holding capacity of the dough (and hence bread yield) and which do not impart stickiness to the dough.

It has now surprisingly been discovered that the improver enzymes (including α-amylase) need not be active within the dough at the mixing stage: their beneficial effects are fully realised even when their activity is delayed until the proving stage or even later (e.g. during the early stages of baking). The inventors have therefore found that the problems associated with stickiness, low water holding capacity and softness at the mixing stage can be avoided if the improver enzymes are mixed in the dough in a latent state and activated during or after the proving stage.

Thus, according to the present invention there is provided a bread improver comprising a latent enzyme preparation.

As used herein, the term "latent enzyme" is intended to define an enzyme which when present as part of a dough mix exhibits a differential activity profile over the course of mixing, proving and baking, its activity during mixing being low relative to its activity during or after proving.

Thus, the latent enzyme preparation may be active during or after proving but relatively inactive during mixing.

Latency may be achieved in a number of different ways. For example, the enzyme may be treated so that it is released into the dough in a controlled fashion, for example during the early stages of proving. Controlled release may be achieved by encapsulation, conveniently

by coating a granular or microparticulate enzyme preparation with a sequestering agent (or capsule). The capsule may take any physical or chemical form, so long as it serves to isolate the enzyme from its substrate(s) in the dough. For example, the capsule may be formed by coating with fat, gelatin or starch (as described infra). Alternatively, the capsule may be formed by surface treating (e.g. by surface irradiation or roasting) particulate enzyme/carrier preparations to create a layer of denatured/inactivated enzyme which delays release of active enzyme into the dough. The level of encapsulation required to effect the invention depends on the particular enzyme, and in someapplications may be below 100% (and in some cases, e.g. when β-amylase is the enzyme, the level of encapsulation may be below 50%). Preferably, however, the level of encapsulation is 50% or higher.

Latency may also be achieved through the use of enzyme preparations in which the enzyme is in close physical association with an inhibitor (for example, in admixture therewith in a binder), so that the enzyme is initially latent due to high local concentrations of the inhibitor but becomes progressively more active as the inhibitor diffuses away (or is broken down in the dough) during mixing. Here, the temporal delay associated with the gradual dilution of the inhibitor effectively provides controlled release of enzyme activity.

A typical inhibitor for use in such embodiments is a pH modulator, such as an acidulant (e.g. ascorbic acid. citric acid and/or sodium diacetate) or alkalifying agent. The choice of pH modulator (i.e. whether an acidulant or alkalifying agent) will depend on the pH-activity curve of the improver enzyme to be made latent. Such pH-activity curves can be established using routine biochemical assays known to those skilled in the art. In preferred embodiments, the pH modulator is an alkalifying agent.

Preferably, the pH modulators for use in the invention are sparingly soluble in the dough mix. This facilitates control of their pH modifying effects in the dough and permits the establishment of pH inhomogeneities arising from a local effect of the pH modulating agent in a microenvironment surrounding the improver enzyme(s) in the dough mix. Suitable alkalifying agents include food acceptable alkali salts, such as calcium hydroxide, calcium carbonate, magnesium hydroxide, sodium hydroxide and sodium bicarbonate.

In a particularly preferred embodiment, the pH modulating agents discussed above are used as a secondary latency system designed to inhibit the activity of any enzyme leaching or escaping from an encapsulant acting as the primary latency system. Such leaching or escape may occur during mixing when the integrity of the encapsulant may be compromised by abrasion during mixing or storage.

Thus, the invention contemplates latent enzyme(s) in which latency is achieved by a primary system comprising an encapsulant (e.g. fat, gelatin or starch) and a secondary system comprising a pH modulating agent.

In yet another approach, latency is achieved by initially providing the enzyme in a physically immobilized form, the enzyme being mobilized over the course of mixing. For example, in preferred embodiments latency is achieved by providing the enzyme to the dough during mixing in the form of an aggregate having a relatively small surface area: volume ratio which gradually increases during mixing due to physical division, so increasing the availability of the enzyme over time. It will of course be appreciated that in such embodiments the form of the enzyme must be selected such that the enzymes are <u>not</u> released immediately upon mixing, but rather is such that latency (and the activity profile discussed earlier) is achieved.

As used herein, the term "enzyme preparation" is intended to cover any preparation of enzyme (howsoever obtained) at any level of purity, so long as the preparation is enzymically active. The enzyme preparations of the invention include preparations exhibiting a plurality of different specific activities, and are conveniently in the form of more or less crude enzyme extracts in admixture with one or more carriers (such as gypsum or maltodextrin).

The enzyme preparations for use according to the invention are preferably in microparticulate or granular form. They are preferably "improver enzymes", which term is used herein to define those enzymes which, when present as part of a bread improver composition, are capable of contributing to its functional properties.

The invention also contemplates a latent enzyme preparation per se for use in the bread improver of the invention, as well as a functional flour per se comprising the bread improver or the latent enzyme preparation of the invention.

Also covered by the invention is a dough or bread comprising the improver, enzyme preparation or functional flour of the invention, as well as bakery concentrates and complete bakery mixes (e.g. bakery dry mixes) comprising various dough ingredients together with the enzyme preparation of the invention.

The present invention is broadly applicable, and the bread of the invention may be of any type, including, white, brown, wholemeal, wheatgerm, malted grain, softgrain, soft rolls, crusty rolls and buns (including tin, crusty and Danish varieties of any of the foregoing). The invention finds particular application in tin breads.

The invention also contemplates a process for preparing a dough comprising the step of: (a) mixing the bread improver or latent enzyme of the invention with flour, yeast, salt and water; or (b) mixing the flour of the invention with yeast, salt and water.

In another embodiment, the invention relates to a process for preparing bread comprising the steps of: (a) mixing a dough (for example the dough of the invention or a dough obtainable by, or produced by, the processes of the invention) in the presence of an improver enzyme; and (b) proving the mixed dough, wherein the action of the improver enzyme is substantially delayed until the proving step (b) (or later, e.g. during the early stages of baking prior to heat inactivation of the enzyme(s)).

The enzyme is preferably selected from any of: amylase, for example α-amylase (e.g. fungal α-amylase); hemicellulase; pentosanase; xylanase; pectinase; pullulanase; other non-starch polysaccharide degrading enzymes; redox enzymes (for example glucose oxidase, lipoxygenase or ascorbic acid oxidase); lipase; protease; δ-mannanase; oxidoreductases (e.g. glucose-oxidase, sulfhydryl-oxidase, SS-isomerase, SS-transferase); carbohydrate-modifying enzymes or combinations of any of the foregoing.

In particularly preferred embodiments, the enzyme(s) are of bacterial, yeast, mammalian or fungal origin. They are also preferably heat resistant or thermostable, for example being deactivated by the baking process but active during proving. The enzyme for use in the invention is preferably: (a) encapsulated (for example such that in use its release into the dough is substantially delayed until proving or post-proving); and/or (b) immobilized during mixing; and/or (c) repressed or inhibited during mixing; and/or (d) activated during or after proving; and/or (e) sequestered during mixing.

In embodiments where the enzyme is encapsulated, the encapsulant is preferably any of: (a) fat; (b) gelatin; (c) gum (e.g. vegetable gum); (d) maltodextrin; (e) starch (e.g. modified starch); (f) emulsifiers; (g) waxes; (h) sugars.

Fats for use in the invention may comprise unmodified and/or hydrogenated and/or fractionated vegetable, animal or marine oils (e.g. tallow, lard, fish, palm, cottonseed or soybean oils). When fat is used as an encapsulant, the fat preferably has a slip melting point of at least about 35°C and/or is provided and/or disposed in sufficient quantities relative to the enzyme that the enzyme is substantially sequestered from the dough during mixing and released during or after proving.

Preferably, the slip melting point is selected to ensure that the fat is substantially solid during mixing at temperatures of from about 15°C to about 30°C and that significant melting (and hence release of enzyme) occurs at temperatures between about 30°C and 35°C (preferably above about 33°C).

It will be appreciated that those skilled in the art will readily be able to determine the appropriate fat characteristics (including slip melting point) empirically for any given set of mixing and proving conditions, by routine trial and error.

Those skilled in the art will realize that the release temperature (and hence the length of time available for released enzyme to act during proving) may vary within a wide range, providing that sufficient enzyme is released for sufficient time to exert a beneficial effect during or after proving. Those skilled in the art are also able to select, by routine trial and error, a fat having a suitable release temperature based on the conditions selected for the proving process.

In most embodiments, the enzyme will also be active for the early part of the baking stage, while the temperature is below that at which the enzyme becomes heat-denatured (about 50°C for fungal α-amylase).

The enzyme may be encapsulated by any suitable process. Many different such processes are known in the art, and include for example (a) spray drying; (b) solvent dehydration; (c) extrusion; (d) air suspension; (e) centrifugal extrusion; (f) coacervation; (g) spray chilling (e.g. as described in EP 0 393 963); (h) fluidized bed coating; (i) combinations of (a)-(h). Particularly preferred is the use of a fat encapsulant applied by spray chilling using methods and apparatus described in EP 0 393 963 (the teachings of which are incorporated herein by reference).

In preferred embodiments, the enzyme is released during or after proving by: (a) temperature-mediated release (e.g. thermal breakdown of an encapsulant); (b) a water-mediated release; (b) an attritional agent (e.g. an enzyme, surfactant or acidulant).

As used herein, the term "attritional agent" is intended to define any agent (for example, a chemical moiety, an enzyme, or a particular physical condition or treatment) which breaks down a barrier between the enzyme and the dough to release the enzyme. In preferred embodiments, the attritional agent is an inherent property of the dough during or after proving, such as its temperature or moisture level.

For example, where the encapsulant is fat, the attritional agent is primarily the temperature differential between the mixing and post-mixing steps: the relatively high temperatures at the proving stage effectively melt the fat capsule and release the enzyme.

Where the encapsulant is starch, the attritional agent may be (at least in part) the water present in the dough, which progressively degrades the starch capsule during mixing and proving to provide timed release of the enzyme (which can be optimized by varying the thickness of the starch capsule).

Where the encapsulant is a pectin gum, the attritional agent may be a pectinase. Other suitable degradative enzymes may be used with other gums (such as guar, xanthan etc.). Timed release of the encapsulated enzyme may then be achieved by controlling the activity of the pectinase, for example by encapsulating it in a fat capsule designed to release the pectinase during or after proving. This system has the further benefit that the gum encapsulant increases the water binding capacity of the dough during mixing. Thus, in this system, the gum capsule is bifunctional, serving both to delay the release of the improver enzyme and to increase the water binding capacity of the dough.

The invention also relates to a process for producing a latent enzyme which is active during or after dough proving but relatively inactive during dough mixing, the process comprising the step of encapsulating the enzyme. The encapsulant may be any of the aforementioned encapsulants and any of the aforementioned encapsulating techniques may be employed.

The invention also contemplates a dough, bread or latent enzyme obtainable by (or produced by) the processes of the invention.

The invention therefore permits *inter alia* higher levels of water to be added to the dough (without producing unacceptable stickiness/softness, so increasing yield. The invention also permits higher levels of improver to be added to the dough at any given level of moisture (so improving loaf volume, crumb colour, softness and keeping qualities and permitting the use of lower grade flours).

The invention will now be described in more detail with reference to examples, which are for illustrative purposes only and are not intended to limit the scope of the invention.

<u>Example 1</u>

Fat (Couva 700™ from Loders Croklaan) was melted and fungal α-amylase (hereinafter FAA) in a gypsum carrier was added in 50:50 ratio with the fat. Couva 700™ is a fractionated,

hydrogenated, refined vegetable fat of non-lauric origin. It has a slip melting point of about 35°C and is hard at room temperature, 42% solid at 30°C and 8% solid at 35°C. The FAA used was Fungamyl BG™ from Novo Nordisk. This is a fungal amylase preparation from Aspergillus oryzae. The enzyme hydrolyzes the α-1,4-glucosidic linkages in amylose and amylopectins forming dextrins and maltose. It contains virtually no side activities, and was used in the form of a free-flowing, non-dusting, agglomerated powder with an average particle size of around 150 microns. The mixture was left to resolidify and was then formed into a coarse powder by passage through a metal sieve.

The encapsulated enzyme powder was included in standard white bread dough at twice the usual level, to magnify the effects of encapsulation. The stickiness of the dough was compared to that of dough with the same amount of standard enzyme added. A marked decrease in dough stickiness was observed relative to the standard enzyme, and once baked the resultant loaves exhibited the full benefits of FAA addition with respect to loaf height and crumb softness.

Example 2

FAA mixed with a gypsum carrier was mixed with molten Couva 700™ with a powder to fat ratio of 1:3. The mixture was spray chilled and formed a fine powder. When incorporated into doughs, a decrease in stickiness was observed in comparison to doughs with equivalent levels of standard FAA present. When 2% extra water was added the dough became soft (but not sticky) and handleability was maintained. In all cases there was no detrimental effect on the increased loaf height or crumb softness.

Example 3

FAA without a gypsum carrier was mixed with molten Couva 700™ with a powder to fat ratio of 1:3. The mixture was spray chilled and formed a fine powder. When incorporated into doughs, a decrease in stickiness was observed in comparison to doughs with equivalent levels of standard FAA present. When 2% extra water was added the dough became soft (but not sticky) and handleability was maintained. In all cases there was no detrimental effect on the increased loaf height or crumb softness.

Example 4

FAA with gypsum and dextrose carriers was mixed with molten Couva 700™ with a powder to fat ratio of 1:3. The mixture was spray chilled and formed a fine powder. When incorporated into doughs, a decrease in stickiness was observed in comparison to doughs with equivalent levels of standard FAA present. When 2% extra water was added the dough became soft (but

not sticky) and handleability was maintained. In all cases there was no detrimental effect on the increased loaf height or crumb softness.

Example 5

Couva 700™ was melted and kept at 40°C. FAA was ground in a mortar and an equal mass of the melted Couva 700™ was added. The mixture was solidified and mixed with a mortar and pestle to form a powder. When incorporated into doughs, a decrease in stickiness was observed in comparison to doughs with equivalent levels of standard FAA present. When 2% extra water was added the dough became soft (but not sticky) and handleability was maintained. In all cases there was no detrimental effect on the increased loaf height or crumb softness.

Example 6

To determine the water uptake of a standard dough when using the encapsulated enzyme system of the invention, the following bread mix was prepared:

Flour (Eng 106)	:	100.0%
Salt	:	2.0%
Yeast	:	2.5%
Water	:	61.0%
Improver*	:	1.0%
Fungal α-amylase	:	200 ppm of 5000 skb or its equivalent in terms of 77000 skb

* The improver contained no enzyme activity.

Mix	:	Tweedy 35, 9.5 watt/hrs, 1 minute delay vacuum, dough temperature 28°C
Scale	:	2lb single piece open top, 5 minutes intermediate proof, 1 hour final proof
Bake	:	240°C, 25 minutes

A farinograph was used to determine the water uptake of the dough, and the loaf height after baking in the oven was also measured. A texture analyser was used to perform a squeeze test to yield a measure of bread softness 3 days after baking. The results show that the enzyme system of the invention increases loaf height and softness whithout reducing water uptake. The data are shown in Table 1, below:

TABLE 1

	Enzyme System Used								
Doughs	25% Flaked	25% Spray Chilled	37% Flaked	50% Flaked	Std FAA	Mean Height	Squeeze	Farinograph No Rest	10 Min Rest
1.	-	-	-	-	+	6.5	555	430	390
2.	-	-	-	-	-	6.1	686	470	440
3.	+	-	-	-	-	6.5	526	470	440
4.	-	+	-	-	-	6.5	619	475	410
5.	-	-	+	-	-	6.3	618	470	430
6.	-	-	-	+	-	6.6	547	460	430
7.	-	-	-	-	+	6.6	548	470	400
8.	-	-	-	-	-	6.2	620	500	470

Example 7

Fat (Revel F™ from Loders Croklaan) was melted and fungal α-amylase (hereinafter FAA) in was added. The FAA was Fungamyl 2500 BG™ 77000skb from Novo Nordisk. The FAA was added in 1:9 ratio to the molten fat, and the mixture spray chilled (to effect spray crystallisation) yielding a fine powder. Revel F™ is a fractionated, hydrogenated, refined vegetable fat of non-lauric origin. It has a slip melting point of about 46°C and is hard at room temperature, 72% solid at 35°C.

CLAIMS

1. A bread improver comprising a latent enzyme preparation which is active during or after proving but relatively inactive during mixing.

2. A latent enzyme preparation for use in the bread improver of claim 1.

3. A functional flour, bakery concentrate or bakery dry mix comprising the bread improver of claim 1 or the latent enzyme preparation of claim 2.

4. A dough or bread comprising the improver of claim 1, the enzyme preparation of claim 2 or the functional flour of claim 3.

5. A process for preparing a dough comprising the step of:
(a) mixing the bread improver of claim 1 or the latent enzyme of claim 2 with flour, yeast, salt and water; or
(b) mixing the flour of claim 3 with yeast, salt and water.

6. A process for preparing bread comprising the steps of:
(a) mixing a dough (for example the dough of claim 4 or a dough obtainable by, or produced by, the process of claim 5) in the presence of an improver enzyme; and
(b) proving the mixed dough,
wherein the action of the improver enzyme is substantially delayed until the proving step (b) or later (e.g. during baking).

7. The invention of any one of claims 1 to 6 wherein the enzyme is selected from any of:
(a) amylase, for example α-amylase (e.g. fungal α-amylase);
(b) hemicellulase;
(c) pentosanase;
(d) xylanase;
(e) pectinase;
(f) pullulanase;
(g) non starch polysaccharide degrading enzymes;
(h) redox enzymes (for example glucose oxidase, lipoxygenase or ascorbic acid oxidase);
(i) lipase;
(j) protease;

(k) combinations of any of (a)-(j),

wherein the enzyme is optionally of bacterial, yeast, mammalian or fungal origin and/or is thermostable.

8. The invention of any one of the preceding claims wherein the enzyme is:

(a) encapsulated (for example such that in use its release into the dough is substantially delayed until or after proving); and/or

(b) immobilized during mixing; and/or

(c) repressed or inhibited during mixing (for example by a pH modulating agent, e.g. an acidulant or alkalifying agent); and/or

(d) activated during or after proving; and/or

(e) sequestered during mixing.

9. The invention of claim 8 wherein the enzyme is encapsulated in any of:

(a) fat (for example unmodified and/or hydrogenated and/or fractionated vegetable, animal or marine oils, e.g. tallow, lard, fish, palm, cottonseed or soybean oils);

(b) gelatin;

(c) gum (e.g. vegetable gum);

(d) maltodextrin;

(e) starch (e.g. modified starch);

(f) emulsifiers;

(g) waxes; or

(h) sugars.

10. The invention of claim 9(a) wherein the fat:

(a) has a slip melting point of at least about 35°C (e.g. 40-50 °C, e.g. about 46 °C); and/or

(b) is provided and/or disposed in sufficient quantities relative to the enzyme that the enzyme is substantially sequestered from the dough during mixing and released during or after proving.

11. The invention of any one of claim 8-10 wherein the enzyme is encapsulated by any of:

(a) spray drying;

(b) solvent dehydration;

(c) extrusion;

(d) air suspension;

(e) centrifugal extrusion;

(f) coacervation;

(g) spray chilling;

(h) fluidized bed coating;

(i) combinations of (a)-(h).

12. The invention of any one of the preceding claims wherein the enzyme is released during or after proving by:

(a) temperature-mediated release (e.g. thermal breakdown of an encapsulant); and/or

(b) a water-mediated release; and/or

(c) an attritional agent (e.g. an enzyme, surfactant or acidulant).

13. The invention of claim 12 wherein the enzyme is a latent fat-encapsulated α-amylase.

14. The invention of claim 13 wherein the α-amylase is encapsulated by spray chilling.

15. The process of claim 6 wherein the action of the improver enzyme is delayed by:

(a) providing the enzyme in a form as defined in any one of claims 8-11, or

(b) releasing the enzyme according to the mechanisms defined in claim 12.

16. A process for producing a latent enzyme which is active during or after dough proving but relatively inactive during dough mixing comprising the step of encapsulating the enzyme, for example:

(a) in any of the encapsulants defined in claim 9 or 10; and/or

(b) by any of the processes defined in claim 11.

17. Dough obtainable by (or produced by) the process of claim 5.

18. Bread obtainable by (or produced by) the process of claim 6.

19. A latent enzyme obtainable by (or produced by) the process of claim 16.

20. A method of increasing the water-holding capacity or decreasing the stickiness of a dough during mixing, comprising the step of adding the bread improver, latent enzyme, functional flour, bakery concentrate or bakery dry mix as defined in any one of the preceding claims.